

# Board of Scientific Counselors

February 24, 2009

Jeff Charles, Ph.D., MBA, DABT

Consulting Toxicologist

# Animal Data Not Sufficient Evidence

- Only 1 species - Mouse
- Only 1 tumor site - Lung
- Clear evidence by only 1 route - Inhalation
- Does not belong to class that causes tumors by genotoxic MOA

# Mouse Data

- Clear evidence by inhalation  
(Cruzan et. al., 2001)
- No more than suggestive evidence by oral  
(4 studies)

# Mouse Oral Studies

- C57Bl6 -GD17 and weekly after weaning at 300 mg/kg - no increase
- O20 - GD17 and weekly after weaning at 1300 mg/kg for 16 weeks - 50% mortality by week 20; all mice severe lung toxicity; increased lung tumors following severe lung toxicity.

# Mouse Oral Studies

- B6C3F1 mix of  $\beta$ -nitrostyrene (30%) and styrene (70%) - styrene doses 0, 204, 408 mg/kg - no increase in lung tumors

# Mouse Oral Studies

- B6C3F1 - 0, 175, 350 mg/kg 78 weeks, obs. 13 additional weeks: male lung tumor incidence = 0, 12, 18%; historical control 12% (0-20%) at Litton.
- NCI conclusion: **Suggestive evidence**

# NTP Approach

- NTP new historical control using Hazleton studies - 4%
- Haseman, et.al. (1984) *Toxicol Path* 12:126-135 (Use of historical controls)
- Haseman, et.al. (1985) *JNCI* 75:975-984 (Corn oil)

# Control Data from NCI Bioassays Performed at Litton or Hazleton from Study Numbers TR000-TR219

	NO. STUDIES	% LUNG TUMORS	AVE. %
<b>Litton 91 Week Studies</b>			
Corn Oil	2	0 0	0
Diet	14	20 6 0 16 20 5 16 11 11 17 5 10 11 5	11
<b>Hazleton 91 Week Studies</b>			
Corn Oil	12	0 0 11 6 0 0 0 18 0 0 11 5	4
Diet	14	5 0 0 0 0 11 0 6 0 0 0 6 5 0	2
<b>Litton 104 Week Studies</b>			
Corn Oil	1	5	
Diet	35	0 25 33 11 20 10 18 15 15 35 45 0 26 10 15 21 5 20 35 20 15 30 35 20 25 20 15 30 35 20 25 20 32 35 20 13 17 8 15 20 27	20
Water Gavage	4	15 10 10 11	11
<b>Hazleton 104 Week Studies</b>			
Diet	7	2 13 4 18 18 12 10	11

# Concurrent Studies in the Same Animal Room

Compound	Missing Animals	Cancer Findings
Nitrilotriacetic Acid (NTA)	18/240	Urinary Tract
Trisodium Salt NTA	0/240	Urinary Tract
Nitrofen	5/240	Liver
p-Nitrosodiphenylamine	2/240	Liver
2-acetyl-aminofluorene	No report issued	Known carcinogen
Amitrole	No report issued	Thyroid; Liver
Styrene	5/240	Lung
$\beta$ -Nitrostyrene	2/240	No convincing evidence of carcinogenicity

# Additional Study Report Comments

## ■ Styrene

- Treated groups received 5 mL/kg b.w. corn oil but controls received 10 mL/kg b.w.

## ■ $\beta$ -Nitrostyrene

- 14 high dose males died during Week 36 due to a handling accident
- Treated groups received 5 mL/kg b.w. corn oil but controls received 10 mL/kg b.w.

# Lung Tumor Incidence in Males (at 91 Weeks)

Compound	Control (%)	Low Dose (%)	High Dose (%)
NTA	20	10	14
Na <sub>3</sub> NTA	10	17	0
Nitrophen	17	0	6
p-Nitrosodiphenylamine	5	22	8
Styrene	0	14	21
β-Nitrostyrene	0	22	6

# Recurrent Comment by Reviewing Pathologists in These Study Reports

“Alveolar/bronchiolar tumors are common in several strains of mice independent of treatment and vary in frequency from study to study.”

# Conclusion

- Increased lung tumors in mice by inhalation; no more than suggestive evidence by oral route
- Draft Substance Profile conclusion of “sufficient” evidence of tumors by two routes of administration is incorrect; RoC listing criteria for “reasonably anticipated” not fulfilled